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Review

Analysis of cancer signaling networks by systems biology to develop therapies

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ABSTRACT

Cancer is a complex and heterogeneous disease, demonstrating variations with respect to tumor types and between individual tumors. This heterogeneity has complicated the search for 'magic bullets'—individual genes or pathways that could be targeted and have beneficial effects for large numbers of patients. Instead, recent studies suggest that cancer can be more effectively analyzed through the use of systems biology techniques that examine multiple pathways and account for interactions between these pathways. In this review, we outline the various ways in which systems biology can be utilized to translate high-throughput data into a signaling network and then computationally analyze how cells make decisions based on the information flow through this network. We then discuss recent studies utilizing network-level analysis to reveal therapeutic targets, predict which tumors will be sensitive to existing drugs, and develop combinatorial therapies that target multiple pathways, demonstrating the potential for systems biology to revolutionize cancer therapy.

1. Introduction

While the search for oncogenic mutations that underly tumor development has revealed many promising candidates [1–3], the question of how to transmit this knowledge into therapeutic approaches remains complex [4]. Given the large number of possible mutations, developing drugs against each oncogene seems impractical; additionally, some mutations appear to be of little consequence, while others play a key role in driving a tumor's development [5]. Further analysis of genetic alterations in tumors has demonstrated that many of the mutated genes reside in key signaling pathways that regulate phenotypes such as proliferation, apoptosis, and migration [2,6]. While it would appear that targeting these pathways rather than focusing on the particular mutation for a tumor would be a promising approach, a strictly pathway-centric interpretation is complicated by the extensive cross-talk between signaling pathways [7]. Therefore, we and others assert that a network-level approach will be the most productive means to uncover new therapeutic options for cancer [8,9]. This methodology considers the flow of information through signaling pathways, the interactions between pathways (through direct protein–protein interaction and transcriptional/translational modification), and the convergence of multiple pathways as cells make phenotypic decisions.

Analysis of the cancer signaling network can be done effectively using tools developed in the field of systems biology [10]. Experimental data for this approach is gathered across multiple pathways and/or of multiple molecular forms (*e.g.*, genomic, transcriptomic, proteomic). Predictive or mechanistic understanding of these large and complex data sets is difficult to elucidate by intuition alone; therefore, experimental studies need to be combined with computational analysis [11]. These computational approaches range in level of mechanistic detail from abstracted models using correlative regression [12] to logic/influence models [13] and finally, to highly specified kinetic models using differential equations [14]. In this review we outline three areas where systems biology approaches are contributing to our understanding of cancer: (1) defining the signaling network, (2) analyzing how oncogenic changes impact signaling and cell behavior, and (3) designing therapeutic strategies.

2. Defining the signaling network

One of the first challenges in taking a network-based approach to studying cancer is to define the components of the cellular network, and more importantly, the manner in which they interact. While the results of the Human Genome Project and Cancer Genome Atlas identified normal and altered genes, characterizing the full protein signaling network is still in the early

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stages for tumor cells and is an active area of systems biology research.

2.1. Identifying components and connections

As a first step to define a protein signaling network, potential protein–protein interactions are experimentally derived from yeast 2-hybrid screens or affinity-based mass spectrometry [15]. In addition, these interactions can be predicted based on sequence or structure information [16]. It is important to remember that protein–protein interactions are highly context dependent and consequently static protein–protein interactomes may have only limited applicability. For example, using an ERK-specific immunoprecipitation antibody with cells treated with epidermal growth factor (EGF) or nerve growth factor (NGF), the specific proteins interacting with ERK were found to be both stimuli and time dependent and these different interactions helped to explain the different cellular responses [17].

To account for this context specificity, two recent studies generated protein–protein interaction networks based on cell specific data. Using protein–protein and protein–DNA interactions from B-cells, Mani et al. [18] analyzed gene expression data from normal and B-cell lymphomas to identify correlation changes in order to identify genes involved in oncogenesis. When lymphomas with known oncogenic mutations were examined by this method, the known oncogene was identified in the top 20 changes by the algorithm. In another study, a protein–protein interaction network was generated using only breast cancer literature and used to analyze 10 published breast cancer gene expression signatures [19]. This analysis determined that these signatures have greater interconnectivity than was previously appreciated since in addition to direct overlap, the genes also share interactions that can be identified in this network.

Methods are also being developed to analyze specific interactions from genomic and phosphoproteomic data sets. Gene expression profiles from 176 gliomas were analyzed by ARACNe, an algorithm that infers transcription factor–target gene interactions, to identify the transcription factors linked to a poor prognosis signature [20]. Focusing on the small number of transcription factors that accounted for most of these signatures, the authors confirmed that C/EBPβ and STAT3 were necessary for the aggressive mesenchymal phenotype in glioblastoma cells. In order to identify the kinase responsible for phosphopeptides identified in mass spectrometry data sets, Linding et al. [21] developed NetworKIN, an algorithm that uses consensus motifs to match the phosphopeptide to a kinase family and then context information such as cell type to narrow down to a specific kinase within that family. This additional information resulted in a 2.5 fold improvement over the use of consensus sequences alone and uncovered novel kinase/substrate predictions in the DNA damage network that were then experimentally confirmed.

2.2. Determining direction and causality

Protein signaling networks are likely to be more informative for studying cancer and developing network-based treatments since they include information beyond interactions, including which protein is upstream and the impact it has on the interacting protein. Protein signaling networks are frequently curated from literature manually [22] or by automatic methods [23], and we will discuss modeling and analysis of these signaling networks in the following section. However, for signaling networks that are well characterized, there can still be a lack of mechanistic detail or quantitative data, preventing straightforward analysis by computational modeling. Two recent papers demonstrate approaches to design experiments that can address these limitations. In the first, the authors developed a method to design experiments that can distinguish between two model topologies [24]. Utilizing a controls approach, they identified inputs (*i.e.*, doses, timings) that will drive each model topology to a set output (*e.g.*, level of phosphorylated ERK). The experimental input that resulted in that target output then identified the correct model topology. In their second report, the authors examined the problem of how to pick optimal experiments to do model calibration for a large mechanistic model [25]. By simulating a large number of experimental conditions they identified a set of complementary experiments that could fit all parameters of a model and demonstrated that no single experiment could perform as well as this network-level approach.

In addition to literature-curated models, several recent studies have highlighted methods to determine the topology of the protein signaling network directly from experimental data. In modular response analysis, the cellular network is reduced to modules and analyzed to determine the extent of individual modules to the global response [26]. While this method has not been applied to cancer signaling networks, it was successfully used to identify differences in feedback in response to EGF and NGF stimulation; with this information the cellular network could be rewired to switch the stimuli-specific responses [27]. Mitsos et al. [28] have recently described a method to optimize logic models to context-specific settings using a training data set and Integer Linear Programming that minimizes the error and number of connections in a pathway. Bayesian nets have been used to develop protein signaling networks from experimental data without utilizing prior knowledge [29,30]. The developed networks were highly accurate, containing both well-known and suspected links that were experimentally verified [30]. Recently, Sachs et al. [31] extended this approach to analyze differences in network wiring between normal and disease states for follicular lymphoma. The authors were able to identify disease-induced network changes in the state of individual proteins and the relationships between proteins. Interestingly, these network-level variations could also be identified between patient samples, potentially providing personalized signaling networks to determine treatment strategies.

3. Analyzing how oncogenic changes impact signaling and cell behavior

Modeling the cellular signaling network can elucidate the roles of individual proteins and how pathways interact to influence cancer progression. Here we review recent studies utilizing computational modeling and experimental validation to profile and characterize three important oncogenic proteins: epidermal growth factor receptor family (ErbB), steroid receptor co-activator (Src), and phosphatidylinositol 3-kinases (PI3Ks).

3.1. ErbB family

The ErbB signaling network is a dynamic and highly interconnected system composed of four trans-membrane tyrosine kinase receptors (ErbB1 (EGFR), ErbB2 (HER2), ErbB3, and ErbB4) that homo- or heterodimerize following ligand binding to activate a variety of downstream pathways [32]. The ErbB family plays an important role in many cancers, with hyper-activation of ErbB signaling resulting from activating mutations, changes in receptor levels, and increases in ErbB ligand production [33]. Not surprisingly given its importance and complexity, the ErbB network has been modeled explicitly or as part of a larger cellular network in a variety of studies (see also a recent review of these modeling efforts, [34]).

Many of these modeling efforts have focused on determining the effects of changes at the receptor level, such as the overex-

pression of ErbB2 found in ~30% of breast cancer patients and overexpression or mutation of ErbB1 found in glioblastoma and lung cancer [32]. A mass-action kinetic model of ErbB1 dimerization and trafficking demonstrated that changes in receptor internalization rate that accompany ErbB1 mutations were sufficient to explain differences in sensitivity to gefitinib [35]. Another modeling study of ErbB1 signaling analyzed the impact of overexpressed or mutated ErbB1 on activation of ERK, and determined that targeting Rap1/B-Raf inhibited ERK activation more effectively than targeting Ras/Raf-1 [36]. This pathway difference is a result of the high concentration of activated receptors at the cell membrane that continue to stimulate the Rap1 pathway over the Ras pathway. To examine the impact of elevated ErbB2, a kinetic model was combined with quantitative experimental studies to examine ErbB1 and ErbB2 dimerization patterns and how this affects ERK signaling [37]. The model found that the transient increase in ERK activity with elevated ErbB2 is a result of the increased number of ErbB1-containing dimers that can be formed with heterodimerization vs. ErbB1 homodimerization only.

ErbB receptor models have also been extended to include all four ErbB receptors, different ErbB ligands, and feedback regulation of downstream signaling events. An expanded model incorporating all four ErbB receptors and both EGF and heregulin (HRG) as ligands demonstrated that increased levels of ErbB2 converted the normally transient EGF-induced signaling into sustained signaling, though changes in ErbB2 level had insignificant effects on HRG-induced signaling [38]. Chen et al. [39] developed a mass-action kinetic model that included all four ErbB receptors, two ErbB ligand classes, and signaling to ERK and AKT. Analysis showed that sensitive parameters were dependent on the choice of ligand (EGF vs. HRG) and signaling output (ERK vs. AKT), and that these output signals were dependent on levels of upstream proteins. With these larger networks, system robustness can be examined and positive and negative feedback loops identified that help to stabilize the signal and to attenuate the signal over time, respectively [40]. For example, using partial least squares regression (PLSR), we have recently identified differences in ErbB1 autocrine loops between cells with mutations in K-RAS or N-RAS [41]. Cells with mutant K-RAS release significantly less TGFα following treatment with inflammatory cytokines, resulting in lower levels of phosphorylated ERK.

In addition to models examining signaling events downstream of the ErbB receptors, recent models have begun to interpret how differences in receptor level and signaling activity are integrated to determine cellular and tumor behavior. Utilizing PLSR to analyze phosphoproteomic data from EGF- or HRG-stimulated human mammary epithelial cells with varying levels of ErbB2, a combination of nine signals were identified that could predict cell proliferation and migration [42]. While this analysis is only correlative at this stage, it suggested that endocytosis and PI3K are important for migration and proliferation. In another approach, a multiscale model of brain tumor progression was developed to examine the role of ErbB1 activation of PLC-γ in the development of novel phenotypes and tumor expansion rate. In the initial mass-action kinetic model, TGFα-induced ErbB1 activation of PLC-γ was shown to influence the switch between migratory and proliferative phenotypes in glioma [43]. Later studies suggested that tumors with higher ErbB1 levels have the tendency to switch from proliferative to migratory dominant phenotype, resulting in enhanced tumor expansion [44]. Building from this initial algorithm, a three-dimensional multiscale agent-based brain tumor was developed to simulate this "proliferation-to-migration" cellular decision process [45]. This three-dimensional model provided new insights, demonstrating that proliferative and migratory cell populations oscillate, impacting the direction and timing of tumor expansion. Recently, another complexity was added to the three-dimensional model by allowing for the development of five distinct clonal populations that have different ErbB1 levels [46]. Simulations found that clones with higher ErbB1 expression have greater expansion rates due to an earlier switch from proliferative to migratory dominant phenotype.

3.2. Src

Src is a member of a family of tyrosine kinases at the center of many signaling cascades and impacts cell behaviors such as cell survival and mitogenesis [47]. Tumors often have elevated Src activity, impacting proliferation, adhesion, and migration, and Src inhibitors are being tested in phase I and II trials [48,49]. Models have been developed to characterize three major mechanisms of Src activation—release of inhibition by C-terminal Src kinase, weakening of the inhibitory intramolecular phosphotyrosine-SH2 interaction, and amplification of a stimulating kinase activity [50]. Through this analysis, conditions were determined in which individual mechanisms were sufficient to overcome auto-inhibition or where cooperation between mechanisms was needed. In a recent kinetic model of Src activation and deactivation, loss of inhibiting proteins or increases in activators through oncogenic changes altered the amplitude and dynamics of active Src [51]. Additionally, elevated levels of Src alter the dynamics of Src activity, resulting in sustained oscillations between active and inactive Src. In a multiscale model of trans-endothelial migration during intravasation, active Src increased the speed of intravasation [52].

Additional modeling studies have examined the interactions of Src with other signaling pathways and in the development and expansion of tumors. In one kinetic model, Src family of kinases was determined to play a central role in the cross-talk between ErbB and insulin signaling [53]. Synergistic activation of ERK was seen at low doses of insulin and EGF due to both direct activation of these signaling pathways and the activation of Src by ErbB1. This Src–ErbB1 interaction may play an important role in cancer behavior. In a logic model of ErbB signaling that was parameterized with transcriptomic, proteomic, and mutational status information from a panel of luminal and basal breast cancer cell lines, clustering identified a mixed cell group that had lost the Src–ErbB1 interactions through oncogenic changes [54].

3.3. PI3K

PI3K is a family of enzymes that phosphorylate the hydroxyl end of phosphoinositides leading to the activation of downstream targets. PI3Ks main downstream mediator is AKT, which opposes apoptosis and influences tumor invasion and metastasis, making PI3K a critical player in cancer progression [55]. PI3K is linked to many cellular functions including cell proliferation, motility, and differentiation and is essential to cell growth and metabolic control [56]. PI3K can be deregulated through activating mutation or loss of phosphatase and tensin homolog (PTEN), a PI3K antagonist [57]. Drugs targeted against the PI3K pathway can suppress tumor growth and tumor angiogenesis and have entered early clinical trials. Studies in xenograft models found that oncogenic Ras was a negative predictor of response to PI3K-inhibitor PX-866 even for cell lines with PI3K mutations [58]. This observation was confirmed in a larger experimental screen that tested 25 PI3K pathway inhibitors and demonstrated that elevated active AKT correlated with sensitivity for many PI3K inhibitors [59]. These studies demonstrate that utilizing PI3K as a therapeutic target will require an appreciation of its role in the broader cellular network.

Many of the previously discussed models of ErbB receptors incorporated PI3K signaling to investigate activation of AKT and cross-talk with RAS/MAPK [35,38,39]. A recent Brownian kinetic model examined how the close localization of RAS and PI3K at cel-

lular receptors facilitates pathway cross-talk [60]. Model results suggested that localization and rate of active RAS release were more important in RAS activation of PI3K axis than the total level of active RAS in the cell. Another kinetic model focused on the cross-talk between PI3K and RAS downstream of platelet-derived growth factor (PDGF) receptor in combination with quantitative experimental data [61]. This study found that PI3K was strongly and directly activated by the PDGF receptor and was relatively insensitive to RAS cross-talk; in contrast, RAS activation of ERK was enhanced by PI3K activity. In addition to these kinetic models, a recent logic model was used to reconcile inconsistencies between the literature and context-specific experimental data about the effects of PI3K [62]. From the literature, PI3K was expected to influence p38/JNK while in experiments with hepatocellular carcinoma cells p38 and JNK activation were found to be insensitive to PI3K inhibition. Through analysis of possible modifications that could explain this contradiction between experiment and the logic model, the authors identified a potential PI3K-independent route to activate p38/JNK through Rac/Cdc42 [62].

4. Designing therapeutic strategies

Cancer has historically been treated by broad-based therapies such as surgery, radiation, and chemotherapy. More recently, molecular-targeted cancer treatments have been developed following a linear strategy focused on single proteins or pathways. Given the complex and non-linear pathology of tumors – both at a cellular and a tumor level – it is perhaps not surprising that these approaches have had only limited efficacy. In addition to improving our understanding of the complex cellular network, a network-level perspective and computational models of the cellular network can help to identify potential drug targets, reveal signatures to identify tumors sensitive to a treatment, and design combinatorial treatments targeted across multiple pathways. While the application of systems biology to these therapeutic questions is still relatively new, several studies have already demonstrated the promise of this approach.

4.1. Modeling to reveal therapeutic targets

While many of the previously mentioned studies suggest potential targets to control cellular behavior, a recent study demonstrates the full power of this approach by analyzing a network model, identifying the mechanism to target, and then developing and testing a monoclonal antibody against the target. Schoeberl et al. [63] utilized an ErbB network model incorporating ligand binding, receptor dimerization/internalization/degradation/recycling, and signaling events through the PI3K axis. Sensitivity analysis determined that ErbB3 was the most important node leading to AKT activation; this was an unexpected result as ErbB3 does not have kinase activity, and is not frequently mutated or overexpressed in tumors. This analysis clearly demonstrated the importance of looking across multiple pathways—while the ultimate result was a single target, the mechanism supporting the importance of ErbB3 in tumor cell proliferation is the combinatorial interaction between multiple ligands and receptors that is influenced by ErbB3. Subsequent to the identification of ErbB3 as a drug target, the group returned to their mass-action kinetic model to find the optimal kinetic and biochemical properties of an inhibitor against ErbB3; this study guided the development of MM-121, a fully human IgG2 monoclonal antibody against ErbB3, which is currently in phase II clinical trials [63].

In addition to identifying novel therapeutic targets, computational modeling can be used to study underlying mechanisms of drug resistance and identify alternative therapeutic strategies. This strategy has been recently explored to find new targets in trastuzumab-resistant ErbB2-positive breast cancer. Trastuzumab (trade name—Herceptin), a monoclonal antibody against ErbB2, is widely used to treat ErbB2 over-expressing breast cancer [64]. Despite trastuzumab's specificity, several studies have shown that high levels of ErbB2 do not always correspond to sensitivity [65,66]. Moreover, tumors have the potential to develop *de novo* resistance toward trastuzumab. A logic model that linked signaling from the ErbB receptors to cell cycle progression identified ERα, c-MYC, CDK4, Cyclin D1, and Cyclin E1 as potential targets in ErbB2 over-expressing, trastuzumab-resistant breast cancer [67]. Interestingly the model results also suggested that targeting multiple ErbB receptors would be less effective than targeting c-MYC, demonstrating the importance of multi-pathway interactions. A mass-action kinetic model of ErbB2/ErbB3 signaling identified the balance between the levels of PTEN and PI3K activating mutation as the dominant factor in resistance to trastuzumab [68]. Quantification of PTEN expression in 122 trastuzumab-treated primary breast tumors showed that increased level of PTEN expression correlated with worse response to the trastuzumab treatment. The importance of multi-pathway balances to determine cellular fate was also recently demonstrated in a model analyzing the differential sensitivity to inflammatory cytokines of K-RAS and N-RAS mutant colon cancer cells [69]. These systems biology studies strongly suggest that combinatorial treatment based on multi-pathway interactions and balances will be an effective and necessary approach to treat cancer.

4.2. Modeling to reveal network signatures that predict drug sensitivity

A shift from conventional cytotoxic agents that halt DNA synthesis in all cycling cells to targeted therapy specific to aberrant pathways in malignant cells is occurring in cancer treatment [70]. Historically, strategies for targeted therapies were guided by the "oncogene addiction" philosophy, in which targeting the mutated proteins unique to the cancer cells was hypothesized to result in the biggest impact [71]. One complication with this linear approach is that single protein predictions have not had universal success; for example, not all ERα-positive breast cancers respond to tamoxifen [72]. New evidence has shown that multi-protein signatures can be more useful than simply relying on mutational status or expression level of one gene to identify subsets of patients with higher responsiveness toward these targeted inhibitors [73]. For example, the combined status of *P53* and *ATM* predicts the clinical response to conventional chemotherapy in breast cancer patients better than either gene alone [74]. In addition to analysis of multiple mutations, proteomic signatures show significant potential to identify subsets of patients that will most likely benefit from targeted therapeutics, and subsequently improve the prognostic value of these agents. As a part of the development of MM-121, a fully humanized monoclonal antibody against ErbB3, a support vector machine algorithm was used to identify a hyper-plane separating responsive from non-responsive cell lines [75]. A multi-protein parameter consisting of ErbB1, HRG, and betacellulin levels was found to best predict cellular response to MM-121.

Several recent studies demonstrate that computational modeling can greatly aid in the discovery and interpretation of these multi-protein signatures for therapeutic response. Statistical models such as PLSR and principal component analysis (PCA) have proven to be highly valuable in identifying network-level molecular signatures corresponding to drug sensitivity. Pritchard et al. [76] applied PCA to time course measurements of the signaling levels of seven phosphoproteins to identify the most relevant phenotypic parameters distinguishing sensitive from resistant cell lines when treated with 17AAG, an HSP90 inhibitor that affects a wide range of proteins. The authors found that a multi-protein signa-

ture consisting of measurements of early phosphorylation of AKT, IKK, STAT3, and p38 following 17AAG treatment was able to distinguish between sensitive and resistant cell lines. A study by Kumar et al. [77] further illustrates the importance of network-level perspective in designing cancer therapy. In this study, the effects of PI3K and MEK inhibitors on migration of ErbB2-overexpressing breast cancer cells were predicted using a PLSR model built from five phosphoprotein measurements—AKT, ERK, p38, and two ErbB1 phosphorylation sites [77]. While these inhibitors have strong specificity for their target proteins, network-level effects were observed due to cross-talk between pathways. As a result of these interactions, the model's ability to predict migration in response to treatment with these inhibitors was lost when only one kinase measurement was used. For example, cells treated with HRG and EGF had different levels of migration despite similar levels of inhibition of AKT, further emphasizing the importance of multi-pathway signatures in predicting drug response.

A variety of studies have identified patterns of gene expression that are predictive of tumor behavior and drug response [78–80]; however, these signatures are of sufficient complexity to prevent intuitive understanding of their meaning in order to find alternative therapies for the non-responsive population [81]. Using Bayesian Factor and Regression Modeling, a recent study demonstrated that large gene expression signatures can be decomposed into a series of smaller signatures that can be more directly linked to a cell signaling module [82]. These modular signatures can predict drug sensitivity, suggesting that with a sufficient number of such signaling module signatures it may be possible to predict which drug or combination of drugs to use from a microarray profile of a tumor.

4.3. Modeling to design combinatorial treatments

The rapid development of targeted inhibitors and the highly heterogeneous nature of tumors have led to an increased interest in designing combinatorial therapeutic strategies [83]. By employing a network-level perspective and targeting multiple pathways simultaneously, it may be possible to overcome pathway cross-talk and redundant mechanisms that are thought to be responsible for the modest responses observed in trials of targeted therapies [84]. Compensation in other pathways in response to treatment with targeted inhibitors has been observed both experimentally and in model simulations. For example, a mass-action kinetic model of IGF receptor signaling in breast cancer cells was able to capture the effect of treatment with a MEK inhibitor [85]. Due to pathway interactions, this resulted in the expected decrease in phosphorylated ERK, but also an unexpected activation of the PI3K/AKT pathways. Observations such as this illustrate the need to simultaneously target multiple pathways for cancer therapy. Additionally, multiscale models such as those developed for the ErbB network may allow for therapeutic strategies to be tested in an *in silico* environment that mimics the cellular heterogeneity of a tumor [46].

Identifying the optimal drug combinations, however, is a challenging and often counter-intuitive process. The countless number of drugs with the potential to be used in combinatorial therapy is overwhelming even with high-throughput screening techniques. For example, identification of a combination of the antipsychotic agent chlorpromazine and antiprotozoal agent petamidine that inhibited the growth of lung tumors in mice required a screen of 100,000 combinations from a set of 600 approved drugs [86]. This example clearly demonstrates that even a small number of compounds quickly yield a very large number of pair-wise combinations, and even more higher-order combinations, particularly if the added variables of dose and time of administration are added. Computational modeling has the potential to accelerate the discovery process and reduce the cost associated with screening for combinatorial hits. A systematic algorithm based on minimal hitting set problems was recently developed to find minimal drug combinations that target a panel of tumor derived cell lines [87]. Using the IC50 values for more than 45,000 compounds tested on the NCI-60 panel, the algorithm identified a combination of fourteen compounds that was able to target all 60 cell lines. In another study, neural network modeling was used to predict the effect of ternary and higher-level combinations from a training set of all pair-wise combinations of six agonists [88]. Although still at a developmental stage, these studies demonstrate the potential of computational modeling to reduce the experimental space one needs to examine in order to identify combinatorial therapies.

In designing combinatorial treatment strategy, it is important to consider possible synergistic and additive effects of multiple drugs. To address this phenomenon, Yan et al. [89] developed an addendum to the well-established Bliss independence criterion to take into account synergistic effects of treatment with two drugs. The group applied their algorithm to TNFα activation of NFκB – a pathway for which single-agent therapy has low efficacy – and found three potential targets for combination therapy (IκB degradation proteasome, HSP90, and IKK-β). The synergistic effect of these three novel targets was simulated using dual-agent combinations and experimentally confirmed. Using ICAM-1 expression as a read-out, treatment using any combination of inhibitors against two of the above targets yielded lower ICAM-1 expression compared to treatment with single inhibitors [89]. Expansion of this algorithm to take into account higher order combinations could contribute significantly to the field of cancer drug discovery.

5. Conclusions

In this review, we have profiled recent studies using systems biology that are improving our understanding of the complex signaling networks in cancer. Through a combination of high-throughput, quantitative experiments and computational tools, systems biology is helping to translate the various 'omes into cellular networks that can be used to identify control nodes that regulate tumor progression. Already, these methods are helping to design novel treatment strategies to overcome the effects of pathway interactions and redundant mechanisms thought to be responsible for the complexity of cancer at a cellular level. This network-level perspective and computational modeling may ultimately help to customize these strategies to the individual patient. An important challenge remaining to employ these network-centric methods more broadly is to develop methods that can analyze more complex situations representative of the tumor microenvironment, including multi-cellular *in vitro* and *in vivo* animal models. This would enable the use of systems biology to analyze the impact of orthogonal treatment approaches used clinically (*i.e.*, drug treatment in combination with surgery or radiation) that impact multiple cell types in the tumor microenvironment.

Conflict of interest statement

The authors declare there are no conflicts of interest.

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